Exploration of Female Fish Ovaries

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Introduction/Hypothesis

- The Multispecies Ovary Tissue Histology Electronic Repository (MOTHER) is a public repository that enables sharing of ovary histology images and metadata for education and research purposes.
- **Question**: Will fish exposed to 17 alpha-ethinylestradiol (EE2) have more effects on their ovaries than fish with no exposure?
  - EE2 is a synthetic estrogen found in birth control pills.
  - It is excreted from the human body and eventually enters waterways that have fish populations.
  - Fish reproductive health may be affected by the EE2 hormone mimic.
  - Our hypotheses were made to explore this question.
    - **1st hypothesis:** If fathead minnows (*Pimephales promelas*) are exposed to EE2, then no differences will be observed on oocyte development compared to non-exposed fish.
    - **2nd hypothesis:** If zebrafish (*Danio rerio*) are exposed to EE2, then no differences will be observed in oocyte development compared to non-exposed fish.

Methods

- We conducted a literature review and identified potential collaborators that exposed fathead minnows or zebrafish to EE2.
- We sent requests via email for their ovary histological images/slides.
- To identify fish oocytes in different stages of development, we adapted QuPath software [1] being used by the MOTHER project to identify monkey ovarian follicles in different developmental stages (see Fig. 1).
  - We edited the original script code and replaced non-human primate follicle classes with the new classes to identify fish oocytes.
- We used an Olympus brightfield microscope and cellSens software to digitize ovary histology slides from California Sheephead (*Semicossyphus pulcher*) and Weedy Seadragon (*Phyllopteryx taeniolatus*).

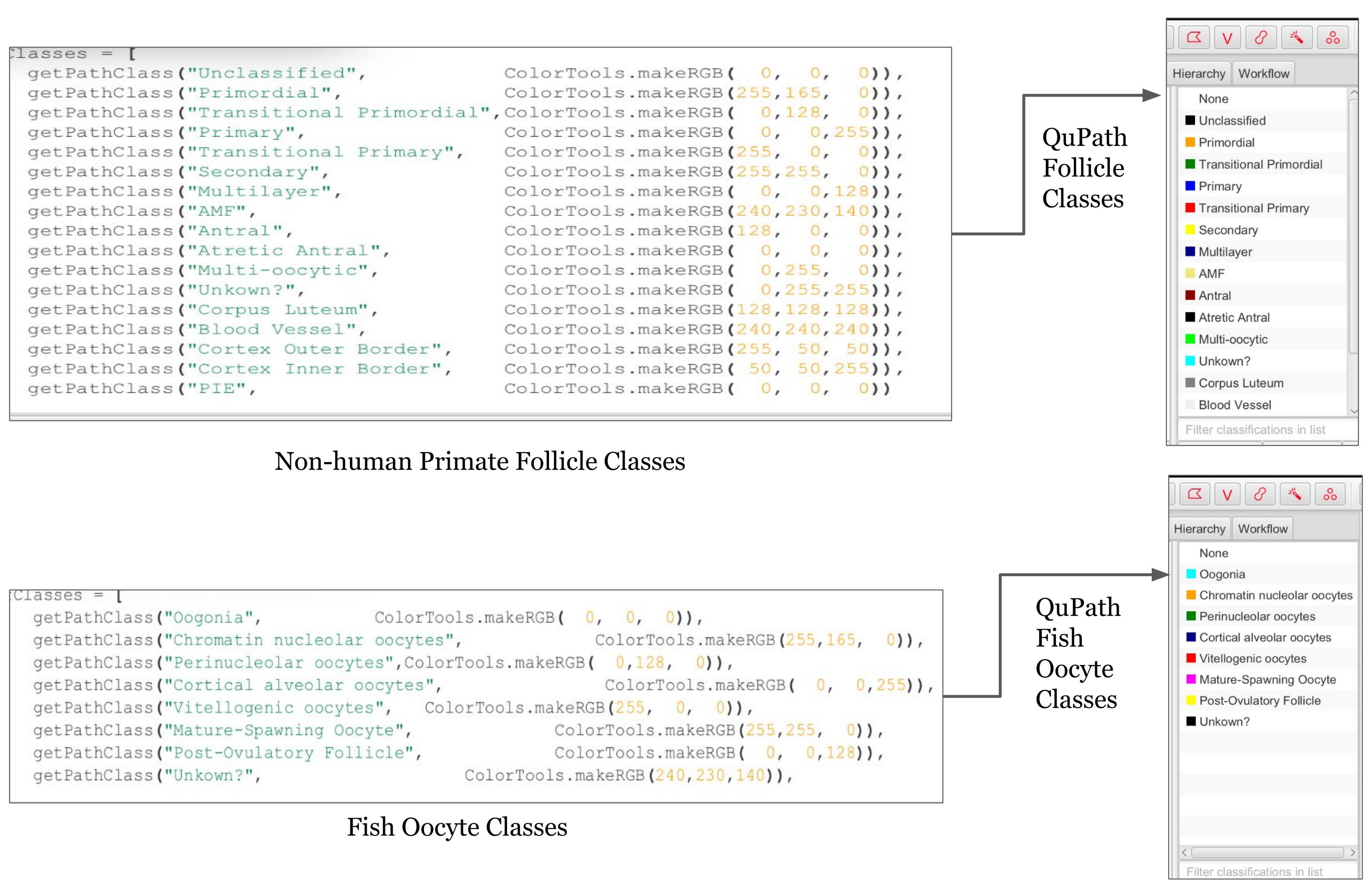

Fig. 1 Adapted QuPath Classification Program. MOTHER project script code (top) and adapted fish oocyte script code (bottom).

Charts/Method

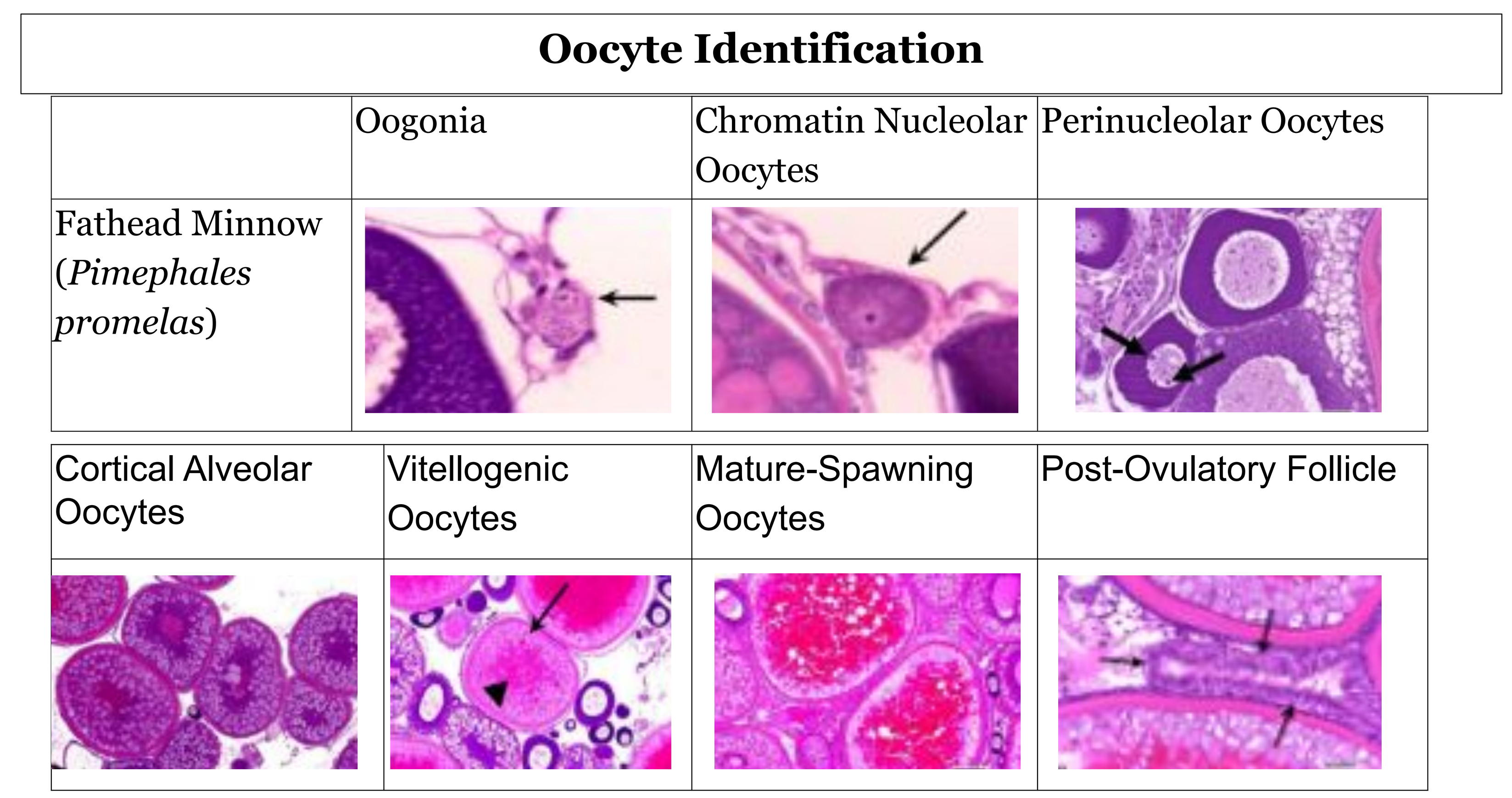

Fig. 2 - A chart showing the different types of oocytes found in fathead minnow. Images and identification adapted from Johnson et al.[2]

Table 1 - Ovary Stages and Variations. This table shows the how ovaries are staged in different species. Fathead minnow, zebrafish, and Japanese medaka from Johnson et al. [2] have the same categorical stages. Stage classifications for the California sheephead were obtained from Sundberg et al. [3], and for the weedy seadragon from Forsgren et al. [4].

Oocyte Stages	Oogonia and Perinucleolar oocytes	Chromatin nucleolar oocytes and perinucleolar	Perinucleolar to cortical alveolar	Vitellogenic oocytes	Late vitellogenic and maturing follicles	Post ovulatory
Fathead Minnow **Zebrafish** **Japanese Medaka** (*Oryzias latipes*)	Stage 0 - Undeveloped	**n/a**	Stage 1 - Early spermatogenic	Stage 2 - Mid-development (Early Vitellogenic) Stage 3 - Late development (Late Vitellogenic)	Stage 4 - Late development /hydrated	Stage 5 - Post Ovulatory
California Sheephead	**n/a**	Immature	Early Maturing (cortical alveolar only)	**n/a**	Mature (Post Ovulatory may also be present)	Regressing /Recovering (Chromatin Nuclear and Perinucleolar may also be present)
Weedy Seadragon	Immature (Oogonia only)	**n/a**	Mature-Non-Reproductive	Mature - Reproductive	**n/a**	**n/a**

Results

- We received two responses to our email requests and follow-up emails to potential collaborators with fish ovary histology slides, but did not receive any histology images in time for this presentation.
- We developed a method for identifying oocytes in fish using QuPath software, see Fig.2 and Table 1, as the MOTHER project had focused on non-human primates and ovarian follicles.
- Using Olympus cellSens Imaging Software and a brightfield microscope, ovary slides of the California Sheephead and Weedy Seadragon were scanned, checked for quality, and documented in a workflow log.
- Using the modified QuPath tool to annotate, we conducted follicle identification of the scanned slides (see Fig. 3).

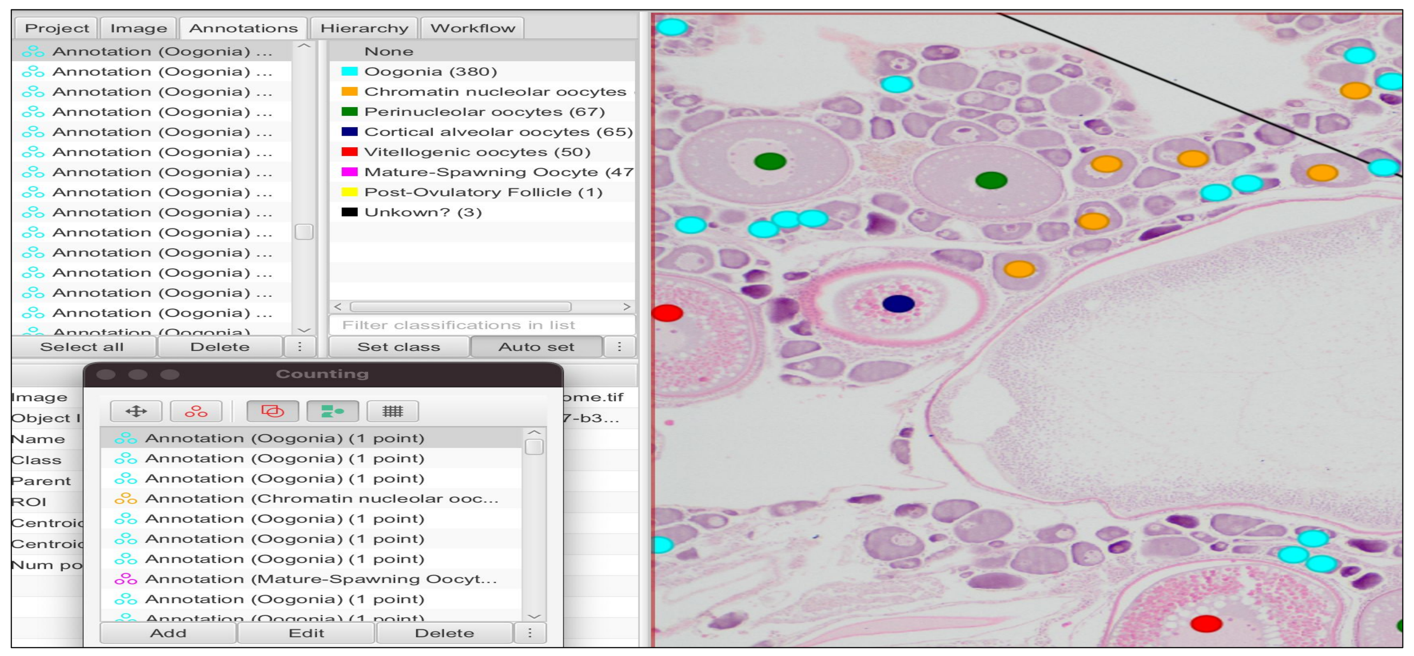

Fig. 3 - Adapted QuPath software with some identified oocytes in California Sheephead histology digitized image. Original slide provided by Dr. Young at California State University, Long Beach.

Future Work

- Determining final counts of identified oocytes
- Determining fish ovary stages
- Identifying oocytes and ovary stages in histology images from our contacted collaborator
- Improving oocyte and stage identification methods for cross species fish investigation.

References

[1] Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P.D....Hamilton,P.W.(2017)..Scientific Reports 7, 16878, doi:10.1038/s41598-017-17204-5.

[2] Johnson, R. & Wolf, J. & Braunbeck, T. (2009).Series on Testing and Assessment: Ecotoxicity Testing: No. 123.

[3] Sundberg MA, L. KA, L. CG, Young KA. Bull South Calif Acad Sci. 2009 Apr;108(1):16-28. doi: 10.3160/0038-3872-108.1.16.

[4] Forsgren, K. L., & Young, K. (2008). Australian Journal of Zoology, 56(6), 441. doi:10.1071/ZO08031.

Acknowledgments

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